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NORMALIZATION OF SPATIAL LEARNING DESPITE BRAIN DAMAGE IN RATS RECEIVING KETAMINE AFTER SEIZURE-INDUCTION: EVIDENCE FOR THE NEUROMATRIX[1]

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Summary.—The concept of the neuromatrix assumes that all behaviours are associated with complex spatiotemporal electromagnetic fields within the brain. The same complex magnetic field can be supported potentially by different mosaics of neuronal associations and result in the same behaviours. In the present study the accuracy of long-term and short-term spatial memory for rats that had been treated with 100 mg/kg of ketamine immediately after the induction of seizures by lithium and pilocarpine did not differ from normal rats despite the conspicuous multifocal neuronal loss. A 30-min. delay of treatment with ketamine resulted in significant memory impairment. Whereas deficits in short-term memory were significantly correlated with cell loss within the CA field of the hippocampus, deficits in long-term memory were related to cell loss within specific thalamic-amygdaloid structures. Implications for the concept of the neuromatrix, if valid, are discussed.

One assumption of the neuromatrix (Persinger, Bureau, & Peredery, 1994) is that all behaviours are correlated with complex spatiotemporal electromagnetic fields within the brain. Functional associations between any number of different brain structures, including unique combinations emerging after partial neuronal loss within multiple brain loci, might be able to support the electromagnetic correlates that result in the display of the *same* behaviour. Recently we have found that different drugs, such as prazosin, acepromazine, propranolol, or ketamine, may satisfy this condition by evoking *specific* and different mosaics of patterns of damage within the brains of rats in which limbic seizures (Honchar, Olney, & Sherman, 1983) were induced by a single systemic injection of lithium and pilocarpine.

This discovery may also be relevant to more profound philosophical questions. For example, can specific electromagnetic patterns that determine the specific behaviours generated through the normal microstructural organization of the brain also emerge within brains containing multifocal, partial neuronal loss? In other words, are there myriads of potential spatial patterns of differential neuronal densities, occluded within normal brain space but expressed by multifocal brain damage, that can serve as alternative substrates

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through which these electromagnetic patterns could operate? The result would be the appearance of "normal" behaviours. The concept would be similar to the fact that a potential difference of "zero" across a neuronal cell membrane would be generated if both the internal and external concentrations of potassium ions were zero ions, one thousand ions, or a billion ions. All measures would appear as "zeros."

Is the brain simply a receptive organ whose structure determines what is detected and what is perceived in space and time? If we change a brain's subtle microstructure by inducing a "critical mass" in the numbers of very local changes in the density of neurons throughout the brain, would properties of extracerebral space and time, to which the normal brain is oblivious, be accessible to this organism? Answers to these traditionally metaphysical questions might be possible by changing the fundamental organization of brain microstructure through a biologically valid experimental manipulation (Persinger, 1999). Unlike the results of single-lesion studies, the type of multifocal and differential neuronal loss subsequent to the inductions of epileptic seizures by lithium and pilocarpine (Peredery, Persinger, Parker, & Mastrosov, 2000), would produce the large numbers of potential combinations required to test these hypotheses.

The present study was designed to discern whether the specific pattern of damage subsequent to treatment by ketamine after the induction of seizures would alter proficiency of learning and memory behaviour in a radial maze. Were previous research replicated (Santi, Cook, Persinger, & O'Connor, in press), then rats seized by lithium-pilocarpine and then treated with ketamine should display normal spatial memory despite the prominent multifocal brain damage. Because we had found an inverse relationship between severity of necrosis and latency of treatment (Peredery, *et al.*, 2000), we also predicted the normalizing effects of postseizure ketamine treatment should decrease as the time between the seizure induction and treatment increased.

Method

A total of 20 male albino Wistar rats, 100 days of age, were injected subcutaneously with 3 mEq/kg of lithium and then 4 hr. later with 30 mg/kg of pilocarpine (Persinger, Makarec, & Bradley, 1988). Because the average onset of rearing, forelimb clonus, and entry into status epilepticus after the injection of the pilocarpine has been about 30 min., the rats were injected either (1) 15 min. before the expected onset (15 min. after the injection of pilocarpine), (2) immediately after the onset of the seizure, or (3) 15 min. or 30 min. after the onset with 100 mg/kg of ketamine (Fujikawa, 1995). Because the focus of this study was to study the effects in the latency of ketamine injection, acepromazine was not injected. Direct comparisons of the effects of these two drugs have been reported elsewhere (Santi, *et al.*, in

press). A group of seized nontreated rats was not included given their extremely high (95%) mortality.

About 14 days later these rats and five normal age-matched male rats (normal rats, employed as controls) were reduced to 85% body weight over a few days. They were then trained and tested in the eight-arm Olton maze for 10 consecutive days. The same four nonadjacent arms were baited with one Fruit Loop while the other four arms were never baited (Santi, *et al.*, in press). The rat was removed from the maze when the food from all four arms had been consumed or 15 min. had elapsed. The numbers of times the rat re-entered an arm containing food that it had entered that day or re-entered a nonbaited arm were defined as working memory (short-term) errors while the numbers of times the rat entered arms that were never baited or did not enter a baited arm were defined as reference (long-term) memory errors.

Within two days of the completion of the learning task the rats were killed by CO_2. The brains were removed quickly and fixed in ethanol-formalin-acetic acid. This method of fixation results in clear cytomorphological detail with minimal artifacts (Persinger, 1979) that does not differ significantly from the details of brains perfused with 10% formalin through the innominate artery. After two weeks the brains were processed for paraffin embedding and microtomed (10 micrometer sections) for coronal sections between the posterior and anterior commissures.

A total of 50 sections, equally spaced between these boundaries, were obtained for each rat. The sections were stained with toluidine blue O and examined by light microscopy at 100X and 400X. The numbers of slides that contained neuronal dropout or pathology for each of the structures designated by Paxinos and Watson (1986) were assessed separately for the left and right sides of the brain. The proportion of sections containing the structure that showed neuronal dropout was the score for that structure. The scores ranged from 0 to 10 (multiplied by 10 for percentage of damage).

The validity of this method has been indicated by the strong correlations (*r*s about .80) between the proportions of cell loss and the quantitative behavioural measures (Harrigan, Peredery, & Persinger, 1991; Persinger, *et al.*, 1994). The correlations between the percentage loss of the numbers of neurons (counted at 1000X) within seized rats relative to normal, age-matched rats for 10 structures (Peredery, *et al.*, 2000) and the proportion of damage employing the methods employed in this experiment for those 10 structures from *different* groups of rats with similar histories have ranged between 0.75 and 0.83. SPSS software was used for statistical analyses.

Results

The means and standard errors of the mean for the average numbers of

working errors, average numbers of reference errors, and total time per day required to complete the task for the 10 days of testing are shown in Fig. 1. There was a statistically significant difference ($F_{4,19}=3.39$, $p<.05$) between the treatments for the averaged time per day to complete the task for all 10 days of testing. *Post hoc* comparisons indicated that the major source of this difference was the longer time required to complete the maze for the group that had received the ketamine 30 min. after onset of status epilepticus compared to the normal rats but not between the normal rats and the other ketamine-injected groups.

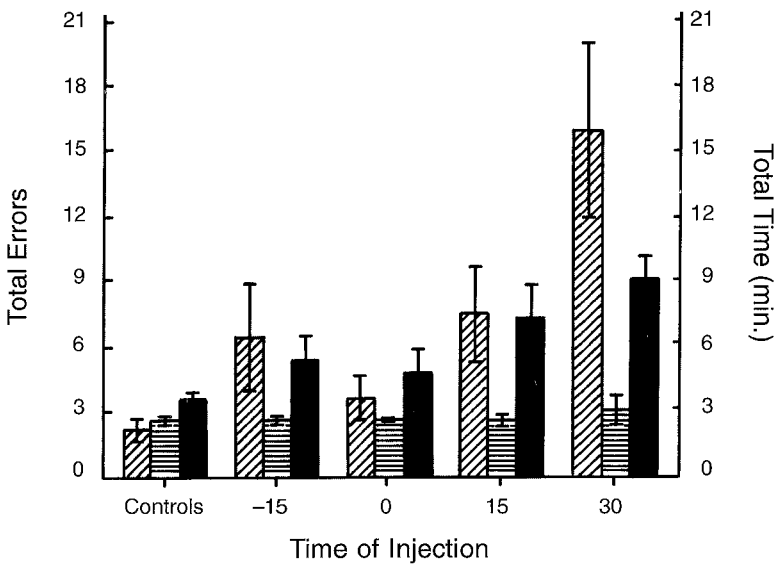

FIG. 1. Average of the total numbers of working errors (diagonal band) and reference errors (horizontal lines) per day (left side) and total time (black bars) before criterion was achieved per day (right side) for control rats or rats that had received ketamine during various times (in min.) before or after the onset of the lithium/pilocarpine-induced seizures. Vertical bars indicate standard error of the mean.

A two-way analysis of variance with one within-subject level (working vs reference errors) and one independent level (normal rats and the four groups of rats injected with ketamine) showed a statistically significant difference ($F_{4,19}=4.56$, $p<.01$) between treatments. In addition to the greater number of working errors than reference errors for all groups ($F_{1,19}=16.62$, $p=.001$), there was a statistically significant interaction between the treatments and the type of error ($F_{4,19}=4.55$, $p<.01$). *Post hoc* analysis indicated that the primary source of the type of error was due to the significantly greater numbers of working errors displayed by the group that received the ketamine 30 min.

after the onset of the seizures compared to all other groups (the controls and the other three ketamine groups) that did not differ significantly from each other. However, there was no statistically significant difference ($F_{4,19} < 1.00$, $p > .05$) among the five groups for the numbers of reference errors.

To ensure that outliers had not distorted the results and to explore further the potential differential effects of the treatments on the learning process, the square roots of the numbers of working errors and of reference errors for each of five successive days or blocks (Days 1 and 2; Days 3 and 4, etc.) were analyzed as a function of the treatment. The difference between the treatments was significant ($F_{4,19} = 4.77$, $p < .01$) and was still due primarily to greater numbers of errors (reference plus working) displayed by the group that had received the ketamine 30 min. after onset of status epilepticus. The interaction between treatment and working vs reference errors was still significant ($F_{4,19} = 5.18$, $p < .01$). The interaction between the types of errors and blocks ($F_{4,76} = 4.57$, $p < .002$) was due to the fewer working errors over the blocks relative to the reference errors that did not change significantly.

The results of the one-way analysis of variance for the proportion of neuronal loss within 41 major clusters of brain structures (Persinger, *et al.*, 1994) as a function of time of the injection of the ketamine demonstrated significant ($p < .01$) differences only for the CA (Cornu ammonis) fields of the hippocampus, the dentate gyrus, and the amygdaloid hippocampal transition. The means and standard deviations for the proportion of damage within the CA fields of the hippocampus for the groups injected with the ketamine 15 min. before the pilocarpine, after the onset of the overt seizures or 15 min. or 30 min. after this display were 8 (9)%, 24 (32)%, 18 (36)%, and 80 (15)%, respectively ($F_{3,16} = 9.70$, $p < .001$; ω^2 estimate = 64%). There was no overlap in the range of scores between the group that received the ketamine 15 min. before the onset of the seizures and the group that received the treatment 30 min. after the onset of the seizures. These values for the dentate gyrus as a function of time of administration of ketamine were 0, 2 (2)%, 5 (8)%, and 62 (45)%, respectively ($F_{3,16} = 7.53$, $p < .01$; ω^2 estimate = 57%).

Post hoc analysis indicated that the primary source of these differences for both structures was due to the greater damage within the CA fields of the hippocampus of the group that received the ketamine 30 min. after the display of overt seizures compared with the other three groups that did not differ significantly from each other. The scores for proportion of damage within the amygdala-hippocampal transition structures for the groups that received the ketamine −15 min., 0 min., +15 min., and +30 min. relative to the overt display of the seizure were 100%, 54 (45)%, 89 (15)%, and 100%, respectively ($F_{3,16} = 4.72$, $p < .01$; ω^2 estimate = 50%). However, the primary source of this difference was due to less damage in rats injected with keta-

mine immediately after the onset of status epilepticus compared to those injected 15 min. before, 15 min. after, or 30 min. after the onset of overt myoclonus.

Dozens of other structures were also damaged but did not display statistically significant time-dependence between the injection of the ketamine and the proportion of damage. In addition to the amygdala-hippocampal transition, the other most damaged structures were the piriform cortex 96 (12)%, the endopiriform nuclei 95 (16)%, and the corticoamygdaloid nuclei 87 (20)%. The five structures least damaged were the cingulate (0%), frontal cortices (0%), orbital cortex (0%), caudate putamen 7 (12)%, and ventral striatum 4 (14)%. The means and standard deviations for the averaged proportion of damage for all structures that involved some discernable neuronal loss were 23% and 12%, respectively, for the left sides of the brain and 23% and 12%, respectively, for the right sides of the brain ($t_{19}=.84$, $p>.05$). The correlation between the damage in the left and right sides of the brain was .95 ($p<.001$).

The means and standard deviations for the structures whose proportions of damage were significantly correlated (Spearman *rho*) with the numbers of working errors, numbers of reference errors, ratio of working to reference errors, or the total time to complete the task during the last five days of testing are shown in Table 1. None of the cerebral cortical structures were significantly correlated with any of these behavioural measures. Only damage within specific regions within the limbic lobe, posterior and lateral thalamus, and medial regions of the thalamus was significantly associated with learn-

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR PROPORTION (%) OF SLIDES WITH NEURONAL DAMAGE AND SPEARMAN *rho* CORRELATIONS BETWEEN THIS DAMAGE AND THE AVERAGE OVER 10 DAYS FOR NUMBERS OF WORKING ERRORS, REFERENCE ERRORS, RATIO OF WORKING/REFERENCE ERRORS (W/R), AND LATENCY TO COMPLETE MAZE

Damaged Area	Damage		Correlation Coefficients (*rho*)*			
	M	*SD*	Working	Reference	W/R	Latency
L-Entorhinal Ctx.	73	34	.58	.14	.60	.52
L-Subiculum	35	35	.58	.36	.65	.59
L-CA Fields	35	38	.48	.50	.54	.57
R-CA Fields	36	40	.60	.57	.62	.63
L-Dentate Gyrus	20	36	.55	.43	.57	.58
R-Anterior Amygdala	23	33	.46	.54	.27	.52
L-Lateral Posterior Thalamus	11	12	.56	.53	.55	.79
R-Lateral Dorsal Thalamus	42	45	.44	.30	.51	.81
R-Intralaminar Thalamus	30	33	.50	.40	.52	.79
R-Midline Thalamus	28	36	.34	.18	.44	.77
R-Mediodorsal Thalamus	32	33	.56	.20	.62	.79

*For all *rhos* $>.45$, $p<.01$.

ing. In all instances the amount of damage was associated with more errors or more time to complete the task. Even though there was no significant difference in the proportion of damage between these structures on the left and right sides of the brain, most of the significant correlations between the amount of damage and numbers of memory errors or maze time involved the proportion of damage within right-sided structures.

Step-wise multiple regression analysis employing the numbers of reference errors and working errors as criterion variables and the measures of damage as the independent variables indicated that only one variable (r = .68, $p < .01$) entered the equation to predict working errors (damage in CA field of the right hippocampus) while three variables [damage in right anterior amygdala, beta = .60; right medial amygdala, beta = –.62; left posterior thalamus, beta = .47, with a multiple $r = .81$ ($F_{3,16} = 10.71$, $p < .01$)] entered the equation for numbers of reference errors.

The zero-order correlations between the proportions of damage within these two clusters of structures and the square root of the numbers of working errors per day ($M = 8.4$, $SD = 7.2$) and the square root of the numbers of reference errors per day ($M = 2.6$, $SD = .4$) are shown in Table 2 in the upper half. All four variables were intercorrelated significantly. However, after partial correlation, when the shared variance with the other two variables was first removed, the residual variance for working errors was significantly correlated only with the amount of neuronal loss within the CA fields of the hippocampus while the residual variance for the reference (long-term memory) was associated significantly with the amount of neuronal loss within the aggregate of structures within the amygdala and posterior thalamus.

TABLE 2

Zero-order Correlations (Above Diagonal) and Partial Correlations* (Below Diagonal) For Relationships Between Errors For Working Memory, Reference Memory, Damage Within the Hippocampal CA Fields of Hippocampus and Composite Damage Within Three Amygdala-Thalamic Structures

	1	2	3	4
1. Reference Errors		.70*	.63*	.81*
2. Working Errors	.19		.68*	.38*
3. Hippocampal Field	–.04	.59*		.27
4. Amygdala-Thalamus	.69*	–.25	.07	

*With shared variance from the other two variables removed.

Discussion

The research by Santi, *et al.* (in press) showed no significant difference between the mean of the proportion of neuronal damage following the injection of ketamine and that following the injection of acepromazine for rats in which seizures had been induced by the same concentrations of lithium (3

mEq/kg) and pilocarpine (30 mg/kg). The scores, however, for proficiency of spatial learning and memory of the rats that had received the ketamine did not differ significantly from those of normal rats, while the rats in which seizures had been induced and which had been treated with acepromazine exhibited marked deficits for memory proficiency within the Olton radial maze. The present study supported these results. The numbers of short-term memory errors, long-term memory errors, and speed of completion of the task displayed by the rats that had been injected with ketamine immediately after the onset of the overt seizures did not differ significantly from normal rats.

The specific neuronal damage associated with the two types of spatial memory, short-term (working) and long-term (reference), within the Olton maze involved different structures. Partial correlations indicated that the numbers of errors committed during working memory were most strongly associated with the amount of damage within the CA fields of the hippocampus. On the other hand, the deficits in long-term (reference) memory were associated with the amount of damage within specific structures contained within the amygdala and thalamus.

The results of this study suggest that the brain might be more optimally conceived as a neuromatrix whose functional elements and relationships between these elements may change after partial damage within *multiple* areas within neuronal space. For some brains, such as those of the rats that had been injected with ketamine, the damage must be pervasive but the behaviours may appear normal given the mosaic of damage and selective sparing of different matrices of neurons. They might support electromagnetic patterns, we have hypothesized, that result in normal behaviours in this particular maze. Alternatively, the ketamine may have selectively spared the structures within the normal matrix of the brain that mediate this specific maze-learning behaviour.

The distinction between the neuromatrix to explain our results and the contemporary concept of "neuroplasticity" is important. The latter concept implies the creation of novel postdamage connections due to compensatory growth of dendritic or terminal axonal processes into compromised regions. The concept of the neuromatrix does not require such growth. Instead, neuroelectromagnetic patterns become "redistributed" through variant mosaics of cells that are produced due to or are unoccluded following the permanent cell loss. Neuroplasticity is not required.

There is an important theoretical implication for this hypothetical approach to the neuromatrix. If not simple learning but also even complex behaviours such as thoughts could be associated with specific spatiotemporal patterns of electromagnetic fields that exist because a specific, subtle neuronal organization supports them, then a different organization of elements

following mild, multifocal neuronal loss might allow the occurrence of these thoughts. Although similar in context, these would not be thoughts due to modification of the individual brain's microstructure due to normal experience. Instead, they would be caused by another functional organization occurring within the neuromatrix that would support the specific electromagnetic pattern that *is* the thought. Because these electromagnetic patterns would be supported by a different physical substrate or mosaic from which different epiphenomenal properties might emerge within the brain, these patterns might also be extremely sensitive to coherence with the information contained within weak, complex, and exogenous electromagnetic fields from the natural and man-made environments.

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